



09876543 : 01290E

RESPONSE TO "SEQUENCE LISTING" REQUIREMENT

Sir:

In response to the Notice to Comply, mailed December 6, 2001, please amend the application as follows:

IN THE SPECIFICATION

Please replace the paragraph beginning at line 20 of page 1 with the following rewritten paragraph:

--A β also referred to as amyloid β peptide (A β P) is a highly aggregating small polypeptide having a molecular weight of approximately 4,500. This protein is a cleavage product of a much larger precursor protein referred to as amyloid precursor protein (APP). The A β protein comprises 39-42 amino acids (SEQ ID NO:1). There are at least five distinct isoforms of APP: 563, 695, 714, 751, and 770 amino

acids, respectively (Wirak et al. (1991)). The A β protein segment comprises approximately half of the transmembrane domain and approximately the first 28 amino acids of the extracellular domain of an APP isoform.--

Please replace the two paragraphs beginning at line 23 of page 4 and ending at line 13 of page 5 with the following two rewritten paragraphs:

--Preferably, the protofibril or compound(s) with protofibril forming ability comprises the following amino acid sequence KLVFFAEDV (SEQ ID NO:2). The A β 1-42 fibrillisation process involves transitional conformation changes from α -helix via random coil to β -sheet. The stable α -helix sequence of residues 16-24 (KLVFFAEDV (SEQ ID NO:2)) apparently plays an important role in this process.

The protofibril or compound(s) with protofibril forming ability may be mutated or modified in relation to corresponding wild-type counterparts. Changes in the KLVFFAEDV (SEQ ID NO:2) sequence will affect the fibrillisation process. For example, changes of the charged amino acids Glu22 and Asp23 into neutral amino acids will induce a random coil structure in the A β peptide. Furthermore, deprotonation of other amino acids such as Asp7, Glu11 and His 6, 13 and 14 in the N-terminal end, has been

Please replace the paragraph beginning at line 24 of page 10 with the following two rewritten paragraph:

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substitution, representing a glutamic acid to a glycine substitution at APP codon 693 (E693G), corresponding to position 22 in the A β sequence. Venous blood was drawn into tubes containing EDTA and DNA was prepared according to standard procedures. SSCP was performed. To sequence exon 17 of the APP gene a 319 bp fragment was amplified with the following primers 5'-CCT CAT CCA AAT GTC CCC GTC ATT-3' (SEQ ID NO:3) and 5'-GCC TAA TTC TCT CAT AGT CTT AAT TCC CAC-3' (SEQ ID NO:4). The PCR products were purified with QIAquick PCR purification kit (Qiagen) prior to sequencing. Direct sequencing was performed in both 3' and 5' direction using the same primers and the BIG Dye cycle sequencing protocol (PE Biosystems) and were then analyzed on an ABI377 automated sequencer (PE Biosystems). The Arctic mutation was seen in one family and not in 56 controls or 254 cases with dementia. Carriers of the arctic mutation showed no vascular symptoms. The mutation was further verified by restriction analysis, since it destroyed a MboII restriction site. The mutation was fully penetrant as no escapees were found. Two-point linkage analysis was performed between the mutation and affection status in the family with an age-dependent penetrance, giving a lod score of 3.66 at recombination fraction 0.00. Two-point lod score was calculated using Mlink from the linkage package (version 5.1) at each of the following recombination fractions

0.00, 0.10, 0.20, 0.30 and 0.40 (q males=q females). A single-locus model with an autosomal dominant inheritance was assumed, which was compatible with the inheritance as it appeared in the pedigree. A cumulative age dependent penetrance was assigned from the known ages of onset in the family. Individuals were put into different liability classes depending on the age at onset (affected) or age at last examination (unaffected). The disease gene frequency and the marker allele frequency were estimated to be 0.001 and the phenocopy rate was set to 0.0001.--

IN THE SEQUENCE LISTING

Please substitute the attached Sequence Listing, numbered as pages 1-2 for the Sequence Listing previously submitted.

REMARKS

1. Applicants hereby submit the following:
- [] a paper copy of a "Sequence Listing", complying with §1.821(c), to be incorporated into the specification as directed above;
 - [XX] an amendment to the paper copy of the "Sequence Listing" submitted on July 9, 2001, the amendment being in the form of substitute sheets;
 - [XX] the Sequence Listing in computer readable form, complying with §1.821(e) and §1.824, including, if an amendment to the paper copy is submitted, all previously submitted data with the amendment incorporated therein;
 - [] pursuant to §1.821(e), reference is made to the computer readable form filed on , in USSN , which presents the identical Sequence information, the use of which is now requested, in lieu of submitting a new computer readable form; and/or
 - [] a substitute computer readable form to replace one found to be damaged or unreadable.

[XX] 2. The description has been amended to comply with \$1.821(d).

3. The undersigned attorney or agent hereby states as follows:

- (a) this submission is not believed to include new matter [\$1.821(g)];
- (b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are believed to be the same [\$1.821(f) and \$1.825(b)];
- (c) if the paper copy has been amended, the amendment is believed to be supported by the specification and is not believed to include new matter [\$1.825(a)]; and
- (d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is believed to be identical to that originally filed [\$1.825(d)].

4. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

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Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences

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by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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The paragraph beginning at line 20 of page 1 has been amended as follows:

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Prefeably, the protofibril or compound(s) with protofibril forming ability comprises the following amino acid sequence KLVFFAEDV (SEQ ID NO:2). The A β 1-42 fibrillisation process involves transitional conformation changes from α -helix via random coil to β -sheet. The stable α -helix sequence

of residues 16-24 (KLVFFAEDV (SEQ ID NO:2)) apparently plays an important role in this process.

The protofibril or compound(s) with protofibril forming ability may be mutated or modified in relation to corresponding wild-type counterparts. Changes in the KLVFFAEDV (SEQ ID NO:2) sequence will affect the fibrillisation process. For example, changes of the charged amino acids Glu22 and Asp23 into neutral amino acids will induce a random coil structure in the A β peptide. Furthermore, deprotonation of other amino acids such as Asp7, Glu11 and His 6, 13 and 14 in the N-terminal end, has been suggested to destabilize the α -helix, leading to initiation of the fibrillation process. Another example is mutations leading to increased immunogenicity in man by using amino acids from mouse A β at specific positions, e.g. Gly 5, Phe10, Arg13. Furthermore, amino acid 13 in A β is known to be part of a heparan sulphate binding motif (13-16; His, His, Gln, Lys) in human, which has been speculated to be involved in AD disease mechanism (inflammation) (Giulian et al. (1998)). In mouse, His 16 is exchanged for Arg 13 destroying the heparan sulphate binding site. Interestingly, mice have never been observed to develop AD. Hence, the use of A β -Arc/Arg13 as an immunogen would be a way to lower possible inflammatory side

effects, elicited with A β peptides with intact heparan sulphate binding motif.

The paragraph beginning at line 24 of page 10 has been amended as follows:

Example 1: Identification of the Arctic mutation

An APP mutation (E693G) in a family from northern Sweden, named the "Arctic" family, is identified, which spans over four generations. The family was screened for mutations in exons 16 and 17 of the APP gene by single strand conformation polymorphism analysis (SSCP) (L. Forsell, L. Lannfelt, (1995)). An abnormal mobility pattern was observed in exon 17. Sequencing revealed an A→G nucleotide substitution, representing a glutamic acid to a glycine substitution at APP codon 693 (E693G), corresponding to position 22 in the A β sequence. Venous blood was drawn into tubes containing EDTA and DNA was prepared according to standard procedures. SSCP was performed. To sequence exon 17 of the APP gene a 319 bp fragment was amplified with the following primers 5'-CCT CAT CCA AAT GTC CCC GTC ATT-3' (SEQ ID NO:3) and 5'-GCC TAA TTC TCT CAT AGT CTT AAT TCC CAC-3' (SEQ ID NO:4). The PCR products were purified with QIAquick PCR purification kit (Qiagen) prior to sequencing. Direct sequencing was performed in both 3' and 5' direction using the

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